NISTTech

Designed Protein Pores as Components for Biosensors

Non-destructively measure single bio-molecules (DNA, toxins, biomarkers) on a single microchip device

Description

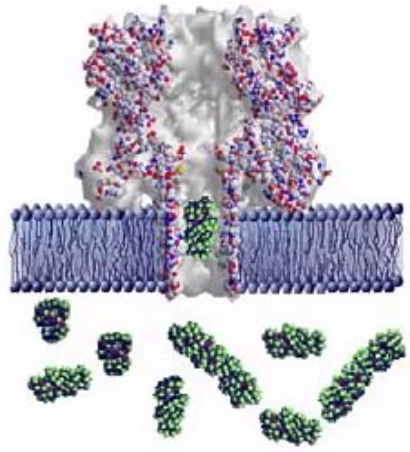
An innovative combination of two patent applications (see abstracts below). Imagine being able to rapidly identify tiny biological molecules such as DNA and toxins (e.g., anthrax) using less than a drop of salt water in a system that can fit on a microchip. Researchers have shown that a single nanometer-scale pore can be used to accurately detect and sort different- sized polymer molecules (a model for biomolecules) that pass through the pore. Traditionally, unknown molecules are measured and identified using mass spectrometry, a process that involves ionizing a large number of target molecules, then analyzing the masses of the resulting molecules to produce a "molecular fingerprint" for the original sample. This equipment can cover a desk. By contrast, the new system is non-destructive, measures one molecule at a time, and can fit in a space as small as a microchip.

The technique involves creating a lipid bilayer membrane similar to those in living cells, and "drilling" a pore in it with a bacterial protein toxin (alpha-hemolysin) designed specifically to penetrate cell membranes. Charged molecules (such as single-stranded DNA) are forced one-by-one into the nanopore, which is 1.5 nm at its narrowest, by an applied voltage. As the molecules pass through the channel, the ionic current flow is reduced in proportion to the size of each individual chain, allowing its size or mass to be easily derived. In one experiment, various-sized polymer chains in solution of the uncharged polymer polyethylene glycol (PEG) were substituted for biomolecules. Each size of PEG molecule reduced the nanopore's electrical conductance differently: larger polymers reduce the current more than do smaller ones. The system is capable of easily discriminating between PEGs whose sizes differ by less than 0.3 nm.

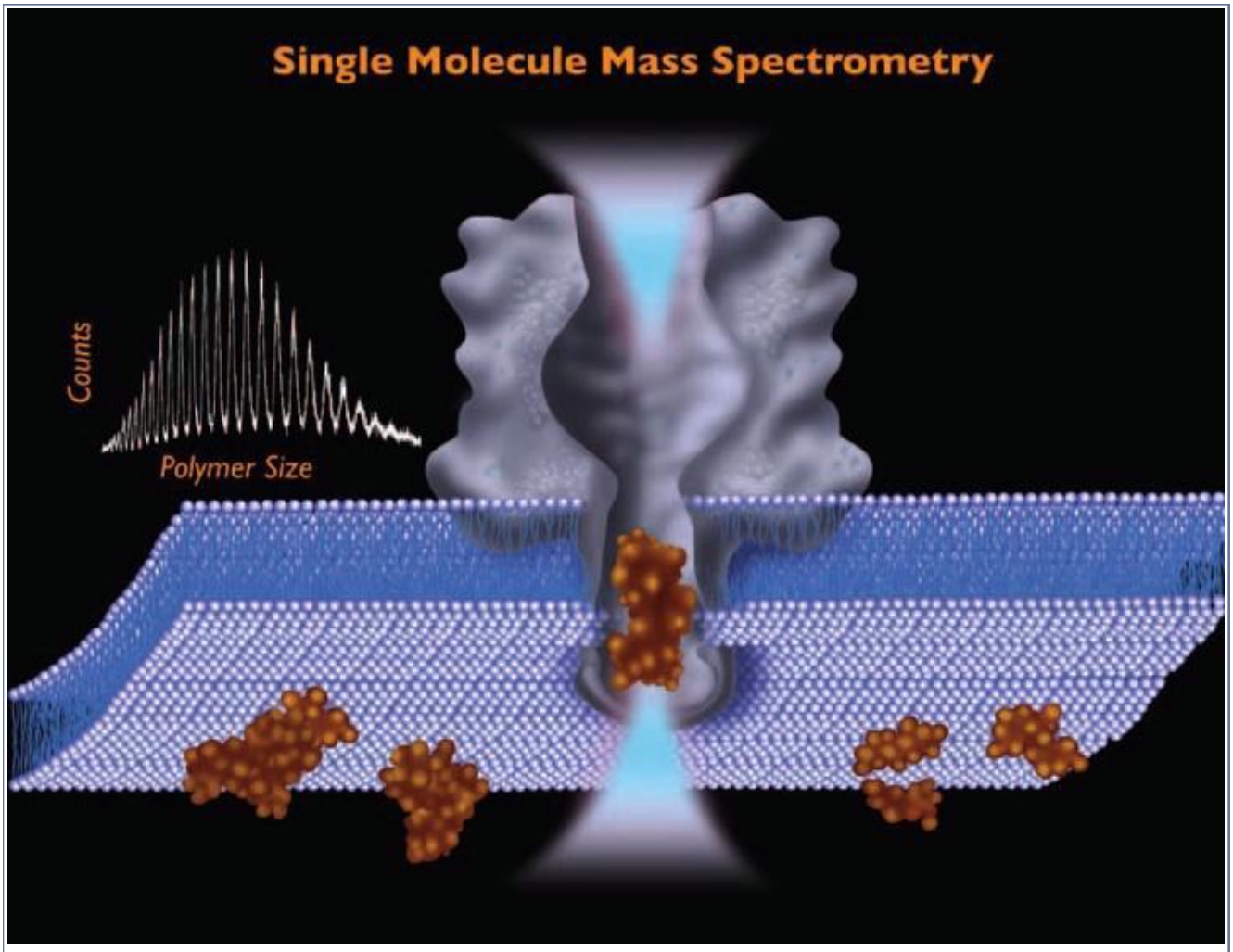
As a control, a solution of a highly-purified PEG of a specific size was characterized with the nanopore. The resulting "fingerprint" closely matched the one identifying samples of the same size polymer in the mixed polymer solution. Further enhancement of the data from the tests yielded mass measurements and identifications of the different PEG sizes that correlate with traditional mass spectrometry.

Because of the nanoscale dimensions involved, the "single-molecule mass spectrometry" technology may one day be incorporated into "lab-on-a-chip" molecular analyzers and single-strand DNA sequencers.

Images



Lipid bilayer membrane (blue) with nanopore; PEG molecules (green globular structure) pass through the pore to the solution on the other side of the membrane.



Molecules entering a nanopore reduce the ionic current through the membrane in proportion to their size.

Applications

- **Molecular footprinting**

Serves the same purpose as mass spectrometry, but only requires one molecule

Advantages

- **Compact**
  Fits onto a microchip
- **Non-destructive**
  Does not destroy the sample
- **Precise**
  Can accurately identify PEG's (polyethylene glycol) with less than 0.3 nm in size difference

Abstract

Method of Stabilization of Functional Nanoscale Pores for Device Applications: Application # 20050191616

A membrane is disclosed made from a compound having a hydrophilic head group, an aliphatic tail group, and a polymerizable functional group. The membrane spans an aperte and may be polymerized. The membrane may be useful for DNA sequencing when the membrane includes an ion channel.

Single Molecule Mass Spectrometry in Solution Using a Solitary Nanopore: Docket # 08-003

The invention consists of a means to measure an electrical current passing through a stable nanopore under an applied voltage while partial occlusion of the pore occurs by molecules that reduce the electrical current because the pore's size is commensurate with the molecules'. The pores may be modified to interact selectively with chosen targets. Specific averaging methods are used that, in effect, act as signal averaging of the individual currents and allows these current levels to be assigned to molecules of different sizes. In addition, the time courses of the chemical interactions of the analytes with the pore can be found once the current levels are assigned. The set of current levels together with the time courses provide a novel two-dimensional method of analysis for charged and uncharged molecules in solution.

Inventors

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Citations

1. O. Braha, B. Walker, S. Cheley, J.J. Kasianowicz, L. Song, J.E. Gouaux, and H. Bayley. Designed protein pores as components for biosensors. Chemistry & Biology, Vol. 4, Issue 7, pp. 497-505, July 1997.
2. J.W. Robertson, c.G. Rodrigues, V.M. Stanford, K.A. Rubinson, O.V. Krasilnikov, J.J. Kasianowicz. Single-molecule mass spectrometry in solution using a solitary nanopore.

Proc Natl Acad Sci U S A, 104(20). pp 8207-11. May 2007.

Related Items

- Article: DNA Sieve--Nanoscale Pores Can Be Tiny Analysis Labs
- Article: Detecting Anthrax Proteins at Ultralow Concentrations
- Article: Tiny Portals to DNA Sequencing
- MERWYN Business Simulation Report

References

- U.S. Patent application #20050191616
- Docket: 05-009US

Status of Availability

This invention is available for licensing.

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